Fish oil supplementation during pregnancy and allergic respiratory disease in the adult offspring

Hansen, Susanne; Strøm, Marin; Maslova, Ekaterina; Dahl, Ronald; Hoffmann, Hans Jürgen; Rytter, Dorte; Bech, Bodil Hammer; Henriksen, Tine Brink; Granström, Charlotta; Halldorsson, Thorhallur I; Chavarro, Jorge E; Linneberg, Allan; Olsen, Sjurdur F.

Published in:
Journal of Allergy and Clinical Immunology

DOI:
10.1016/j.jaci.2016.02.042

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

Fish oil supplementation during pregnancy and allergic respiratory disease in the adult offspring

Susanne Hansen MSc[1], Marin Strøm PhD[1], Ekaterina Maslova ScD[1], Ronald Dahl MD, DMSc[2,3], Hans Jürgen Hoffmann PhD[2], Dorte Rytter PhD[4], Bodil Hammer Bech MD, PhD[4], Tine Brink Henriksen MD, PhD[5], Charlotta Granström MSc[1], Thorhallur I Halldorsson PhD[1,6,7], Jorge E Chavarro, MD, ScD[8,9,10], Allan Linneberg MD, PhD[11,12,13], Sjurdur F. Olsen MD, DMSc[1,8]

[1]Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark
[2]Department of Pulmonary Medicine and Allergy, Aarhus University Hospital, Aarhus, Denmark
[3]The Allergy Centre, Odense University Hospital, Odense, Denmark
[4]Department of Public Health, Section for Epidemiology, Aarhus University, Denmark
[5]Department of Pediatrics, Aarhus University Hospital, Skejby, Denmark
[6]Faculty of Food Science and Nutrition, University of Iceland, Reykjavik, Iceland
[7]Unit for Nutrition Research, Landspitali University Hospital, Reykjavik, Iceland
[8]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[9]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[10]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA
[11]Research Centre for Prevention and Health, the Capital Region of Denmark, Copenhagen, Denmark
[12]Department of Clinical Experimental Research, Glostrup University Hospital, Glostrup, Denmark
[13]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Corresponding author:

Susanne Hansen

Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark

Phone: +45 32684581, email: suu@ssi.dk

Declaration of all sources of funding:

The project described was supported by Grant Number R21AT004603 from the National Center For Complementary & Alternative Medicine. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Complementary & Alternative Medicine or the National Institutes of Health. This study was furthermore funded by the Danish Agency for Science, Technology and Innovation [DSF: 09-067124 (Centre for Fetal Programming), DSF: 09-063072 (Danish Obesity Research Centre), DFS: 2101-06-0005, FSS: 09-065631], the Lundbeck Foundation (R13-A907), and the Danish Lung Association (2-2010). The funding sources had no role in the study.

ABSTRACT

Background: Maternal supplementation with long chain n-3 polyunsaturated fatty acids (PUFAs) may have immunological effects on the developing fetus through several anti-inflammatory pathways. However, there is limited knowledge of the long-term programming effects.

Objective: In a randomized controlled trial (RCT) from 1990 with 24 years of follow-up, our aim was to determine if supplementation with 2.7 g long chain n-3 PUFAs in pregnancy can reduce the risk of offspring asthma and allergic rhinitis.

Methods: The RCT included 533 women who were randomly assigned to receive fish oil during the 3rd trimester of pregnancy, olive oil, or no oil in the ratio 2:1:1. The offspring were followed in a mandatory national prescription register with complete follow-up for prescriptions related to the treatment of asthma and allergic rhinitis as primary outcomes. The offspring were furthermore invited to complete a questionnaire (74% participated) and to attend a clinical examination (47% participated) at age 18-19 years.

Results: In intention-to-treat analyses, the probability of having had asthma medication prescribed was significantly reduced in the fish oil group compared to the olive oil group (HR=0.54, 95% CI:0.32-0.90, p=0.02). The probability of having had allergic rhinitis medication prescribed was also reduced in the fish oil group compared to the olive oil group (HR=0.70, 95% CI:0.47-1.05, p=0.09), but the difference was not statistically significant. Self-reported information collected at age 18-19 years supported these findings. No effects were seen on lung function or allergic sensitization at 18-19 years of age.

Conclusion: Maternal **s**upplementation with fish oil may have prophylactic potential for long-term prevention of offspring asthma.

Key messages

- In this randomized controlled trial with 24-years of follow-up, maternal supplementation with fish oil reduced the risk of asthma medication use in the offspring compared to a placebo group that received olive oil.
- Maternal supplementation with fish oil was, however, not associated with allergic sensitization or lung function at 18 years of age.
- This advocates for a potential role of long chain n-3 PUFAs in preventing offspring asthma in a long term perspective.

Capsule summary

Results from this randomized controlled trial with 24 years of follow-up support the hypothesis that supplementation with long chain n-3 polyunsaturated fatty acids in pregnancy may have prophylactic potential for preventing offspring asthma.

Key words

Randomized controlled trial, fetal programming, asthma, allergies, n-3 polyunsaturated fatty acids

Abbreviations

ATC: Anatomical Therapeutic Chemical classification system

BMI: body mass index

CI: confidence interval

DHA: docosahexaenoic acid

DNPR: Danish National Patient Registry

ECP: eosinophil cationic protein

EPA: eicosapentaenoic acid

FEV_1: forced expiratory volume in the first second

FVC: forced vital capacity

HR: hazard ratio

ICD: International Classification of Diseases

IgE: immunoglobulin E

IQR: interquartile range

OR: odds ratio

PUFA: polyunsaturated fatty acids

RCT: randomized controlled trial

RMPS: Register of Medicinal Product Statistics

SD: standard deviation

INTRODUCTION

There is increasing evidence that environmental factors in fetal life may impact the individual's susceptibility to develop asthma and allergic diseases.[1] Particular focus has been on maternal intake of the long chain n-3 polyunsaturated fatty acids (LCn3PUFAs), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), abundant in seafood and fish oil. EPA and DHA may affect the function of the fetal immune system through several anti-inflammatory mechanisms resulting in a reduced T_H2 allergic immune response.[2] This has been substantiated in two previous randomised controlled trials (RCTs) with fish oil supplementation leading to reduced formation of cytokines produced by T_H2 cells at birth.[3-5] Fish oil supplementation furthermore had a beneficial effect on skin sensitization and immunoglobulin E (IgE) related food reactions and eczema in infants up to 12 months.[6] In 2008, Olsen et al. published results from the first randomised placebo controlled trial that investigated the long-term effect of fish oil supplementation in late pregnancy and they reported a beneficial effect on offspring asthma discharge diagnoses from a mandatory national patient register.[7] However, since only patients seen in hospitals are reported to the patient register[8], asthma discharge diagnoses from this register are known to underestimate the true occurrence of asthma in the general population.[9]

In the same RCT, we therefore decided to extend the analyses with methods of asthma and allergic rhinitis ascertainment that are more relevant for the general population, and with follow-up up until 24 years of age. Our aim was to determine whether our previously reported reduced occurrence of asthma discharge diagnoses in offspring whose mother had taken fish oil in pregnancy could be extended to a more varied phenotype that also included mild and moderate manifestations of allergic respiratory disease and may be more important for disease prevention on a population level.

METHODS

Study cohort

The Aarhus Trial recruited 533 Danish pregnant women (61% of those eligible) with singleton pregnancies through antenatal care clinics in 1990. The women were block-randomised in the ratio 2:1:1 stratified by parity to three groups that received a daily supplementation of either fish oil, olive oil, or no oil from gestational week 30 until delivery. The study design and the original aim have been described in detail elsewhere.[10] Briefly, 266 women were randomised to the intervention group and received four 1g gelatine capsules with fish oil (Pikasol®: 32% EPA, 23% DHA and 2 mg tocopherol/ml)) daily, corresponding to 2.7 g LCn3PUFAs per day. An additional 136 women were randomised to the placebo group and were given four similar looking 1 g capsules with olive oil (72 % oleic acid (18:1n-9) and 12 % linoleic acid (18:2n-6)) per day. Women allocated to the two oil capsule groups and the study coordinators were blinded to treatment allocation. A third group of 131 women were randomised to receive no oil capsules but were informed about the purpose of the trial and about the potential beneficial effects of supplementation with n-3 fatty acids, thus acting as a passive intervention positive control. Active fish oil supplementation was associated with longer gestations[10] when compared against placebo, and it raised maternal EPA-derived thromboxane and prostacyclin production[11], and increased the concentrations of long chain n-3 PUFAs in umbilical blood and tissues[12] and in the early breast milk[13] when compared against a combination of both control groups.

In 2008-2009, a follow-up of the 18-19-year old offspring of originally enrolled mothers was undertaken. At that time, 517 (97%) mother-child dyads were still alive and living in Denmark. An overview of the original trial and the follow-up is presented in Figure 1.

The study was approved by the local ethics committee (M-ÅA 20060182) and the Danish Data Protection Agency (journal no. 2006-41-6257), and all participants gave written consent. The Aarhus Trial is registered under NCT01353807.

Register-based outcomes

We assessed cases of asthma medication use from the national prescription register that holds information on all prescriptions filled in Danish pharmacies written by doctors from all levels of the health care sector.[14] This allows for complete follow-up for all individuals remaining within the

country over their lifetime. We were able to follow-up 522 of the 533 individuals born to women in the trial (98%); the remaining 11 individuals were lost to follow-up due to emigration. We used Anatomical Therapeutic Chemical classification system (ATC) codes R03A, R03B, R03C, and R03D for asthma and ATC codes R01AC, R01AD, R01AX, R06A, S01GA, and S01GX for allergic rhinitis. We defined cases of asthma medication using a modified validated definition[15] as those who had filled ≥ 2 prescriptions for beta-2-agonists or steroids or ≥ 1 prescription for leukotriene receptor antagonists. Allergic rhinitis cases were defined as those having filled ≥ 2 allergic rhinitis prescriptions including eye drops, nasal decongestants, and oral anti-histamines. The capture time was from the start of the register in 1995 until the end of 2014.

As in our previous report,[7] we also identified individuals with a discharge diagnosis of asthma from the national patient register. Follow-up was extended by 7 years relative to our previous report, through the end of 2013. Briefly, we used International Classification of Diseases (ICD) 8 and ICD-10 codes 493.00, 493.01, 493.02, 493.08, 493.09, J45.0, J45.1, J45.2, J45.8, J45.9. Hospital diagnoses of allergic rhinitis were not included as an outcome due to few cases (n=9, 1%).

Clinical outcomes and biomarkers

We performed lung function testing and obtained blood samples from 243 individuals (46%) who participated in a follow-up clinical examination at age 18-19 years in 2008-2009. Serum total IgE, specific IgE against 12 common inhalant allergens, and eosinophil cationic protein (ECP) were quantified by fluoroimmunoassay using ImmunoCAP (Phadia Laboratory Systems AB, Uppsala Sweden). 'Allergic sensitization' was defined as a positive test for specific IgE (≥0.35 kU_A/L) to at least one of the 12 allergens. A cutoff of ≥1.00 kU_A/L was also examined. Based on sensitization status (≥0.35 kU_A/L), we furthermore examined phenotypes of allergic asthma (asthma medication use with sensitization) and non-allergic asthma (asthma medication use without sensitization). Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured using a daily calibrated Vitalograph spirometer (Vitalograph, Ireland). From these measurements we calculated the ratio of FEV_1/FVC and the FEV_1 in % of predicted value (FEV_1%predicted) (based on values from Qaunjer et al.) [16] as continuous variables.

Self-reported outcomes

In 2008, at ~18 years of age, 382 offspring (72%) completed a self-administered web-based questionnaire with four questions on asthma and hayfever that followed the International Studies on Asthma and Allergies in Childhood core questionnaire.[17] Based on answers to this questionnaire we identified individuals reporting 'Ever doctor diagnosed asthma', 'Ever doctor diagnosed hayfever', 'Current asthma medication use', and 'Current hayfever symptoms'.

Analytical strategy

We decided a priori to base the evaluation of the effects of fish oil on the comparison between the fish oil group with the olive oil because these groups consisted of a double blind administration of oil capsules. Our main analyses therefore included 394 mother-offspring pairs; 260 from the fish oil group and 134 from the olive oil group. This decision is supported by data collected postpartum and discussed in detail elsewhere[7] showing that the randomization between the two blinded arms worked well, that olive oil was provided in isocaloric amounts to fish oil, and that the dose of olive oil could reasonably be assumed to be inert in relation to the outcome under study. In contrast, the no oil group was unblinded, was informed of the objectives of the study and of the potential health benefits of fish consumption, and had comparable effects on gestation length to the active intervention arm in the original trial.[10] We therefore considered the no oil arm to represent the effect of a passive intervention consisting of nutritional advice. Comparisons of outcomes between the no oil arm and the active intervention (fish oil) and control (olive oil) arms are presented in Supplementary Tables I-III.

Cumulative incidence curves were generated using the Kaplan-Meier method. In primary intention-to-treat analyses, Cox proportional hazards regression analysis was used to estimate the effect of fish oil relative to that of olive oil expressed by hazard ratios (HR) and 95% confidence intervals (CI); age was used as the underlying time scale. In the national patient register, we followed offspring from birth in 1990 until first asthma discharge diagnosis, death, or end of follow-up in 2013. In the prescription register, we followed offspring from 1995 when data became available until first occurrence of medication cases, death, or end of follow-up in 2014. The proportional hazards assumption was evaluated by visual inspection of residual plots and by a Wald test for interaction between time and treatment group and was fulfilled in all analyses.

In secondary analyses, we used logistic regression models to evaluate the effect of fish oil on self-reported and clinical binary outcomes and calculated odds ratios (OR) and 95% CI. For continuous outcomes, we used linear regression models to estimate the mean difference or ratios between geometric means and 95% CIs. Due to the loss to follow-up in these analyses, we could not apply the intention-to-treat principle, and we therefore decided a priori to adjust for offspring sex and maternal smoking as recorded in the interview at enrolment in gestational week 30, since these were the covariates strongest associated with the outcomes in our data.

The following sensitivity analyses were decided upon a priori: stratification by offspring sex, and adjustment for birth weight and gestational age to investigate if they could explain the potential impact of fish oil on the outcomes.

All tests were 2-sided and a p-value of <0.05 was considered to be significant. Analyses were performed by SAS software package, version 9.3.

RESULTS

As reported earlier[10], the original randomization worked successfully and created similar trial groups with respect to the distribution of covariates (data not shown). When we examined the prevalence of maternal and paternal asthma and hayfever history from the questionnaire completed by the offspring in 2008, no differences between the trial groups were seen (data not shown).

Register-based outcomes: The probability of having had asthma medication prescribed was significantly reduced in the fish oil group compared to the olive oil group (HR=0.54, 95% CI: 0.32-0.90, p=0.02;Table I, Figure 2b). For our analyses based on the patient register we likewise found a reduced probability of asthma diagnosis in the fish oil vs olive oil group (HR=0.31, 95% CI: 0.13-0.75, p=0.01; Table I, Figure 2a), which is in agreement with our previous evaluation with follow up until age 16 years. The probability of having had allergic rhinitis medication prescribed was also reduced in the fish oil group compared to the olive oil group, but this difference was not statistically significant (HR=0.70, 95% CI: 0.47-1.05, p=0.09; Table I, Figure 2c). Passive intervention had similar effects to active intervention (supplementary tables I-III).

Table I.

Registry-based outcomes (n=396)	**N cases (%)**	**Hazard ratios (95% CI) Crude**
Asthma discharge diagnosis		
Fish oil	8 (3%)	0.31 (0.13-0.75)
Olive oil	13 (10%)	1.00
		p=0.01
Asthma medication		
Fish oil	31 (12%)	0.54 (0.32-0.90)
Olive oil	28 (21%)	1.00
		p=0.02
Allergic rhinitis medication		
Fish oil	57 (22%)	0.70 (0.47-1.05)
Olive oil	40 (30%)	1.00
		p=0.09

Hazard ratios (HRs) and 95% confidence intervals (CI) of register outcomes of asthma and allergic rhinitis up to 24 years of age in the fish oil group relative to the olive oil group. Hazard ratios calculated using Cox regression analysis.

Self-reported outcomes, clinical outcomes, and biomarkers: We observed few differences between mother-offspring pairs who participated in the follow-up and those who did not (Table II), but the participation in the clinical examination differed across randomization groups, and was lower in the fish oil group (44 vs. 55%). In the sample of offspring with self-reported data (Figure 3a, supplementary Table IV), we found lower odds of current asthma medication use (OR=0.34, 95% CI: 0.12-0.99, p=0.049) in the fish oil group compared to the olive oil group after adjustment for a priori selected confounders. For doctor diagnoses of asthma and hayfever and current hayfever symptoms, odds were also lower in the fish oil group, but these differences were not statistically significant. We did not observe any significant differences between the fish oil and the olive oil groups in lung function measurements, total IgE or specific IgE, or ECP among individuals presenting to the clinical evaluation (Figure 3b and 3c, supplementary Table IV). Allergic sensitization with the secondary cutoff of 1.00 KU_A/L appeared to be less frequent in the fish oil group compared to the olive oil group (OR=0.56, 95% CI: 0.28-1.13, p=). When examining asthma phenotypes, there was a significant effect on allergic asthma (OR=0.27, 95% CI: 0.08-0.93, p=), whereas there was no significant effect on non-allergic asthma (OR=0.47, 95% CI: 0.16-1.32, p=); although both estimates were in the same direction.

Stratification by offspring sex did not indicate differential effects for boys and girls (data not shown). Birth weight and gestational age did not explain the effect of fish oil on the outcomes (supplementary Table V).

Table II. Characteristics of mother-child pairs in the Aarhus Trial

	Clinical examination			Questionnaire		
	Participants n=243	Non-participants n=274	p	Participants n=382	Non-participants n=135	p
Mother						
Randomization code			0.04			0.51
Fish oil	108 (44%)	152 (55%)		188 (49%)	72 (53%)	
Olive oil	72 (30%)	62 (23%)		104 (27%)	30 (22%)	
No oil	63 (26%)	60 (22%)		90 (24%)	33 (24%)	
Age (years)	29.8 (4.4)	28.9 (4.4)	0.03	29.7 (4.3)	28.5 (4.4)	0.01
Parity (% nulliparous)	142 (58%)	160 (58%)	0.53	224 (59%)	78 (58%)	0.59
Smoker (% non-smoker)	176 (72%)	180 (66%)	0.11	281 (74%)	75 (56%)	<0.001
Fish intake (% low intake)	46 (19%)	59 (22%)	0.76	76 (20%)	29 (21%)	0.22
Pre-pregnancy BMI (kg/m^2)	21.0 (19.7-22.7)	21.3 (20.1-23.2)	0.16	21.1 (19.8-22.7)	21.3 (20.0-24.0)	0.14
Offspring						
Female (% girls)	136 (56%)	100 (37%)	<0.001	194 (51%)	42 (31%)	<0.001
Birth weight (g)	3594.7 (458.8)	3485.4 (510.6)	0.01	3551.8 (500.2)	3494.3 (595.0)	0.25
Gestational age (days)	283.0 (10.6)	281.4 (12.0)	0.10	282.3 (11.3)	281.6 (11.6)	0.54
Asthma medication use	36 (14.8%)	35 (12.8%)	0.50	52 (13.6%)	19 (14.1%)	0.89
Asthma discharge diagnosis	11 (4.5%)	13 (4.7%)	0.91	15 (3.9%)	9 (6.7%)	0.19
Allergic rhinitis medication use	58 (23.4%)	65 (23.7%)	0.97	38 (28.2%)	85 (22.3%)	0.17

Values are mean (SD), median (25^{th}-75^{th} %), or n (%).

P-values calculated using Chi-square tests, t-tests, or Wilcoxon's rank sum-tes

DISCUSSION

Fewer children in the fish oil group compared to the olive oil group had been prescribed asthma medication during the 24-year follow-up period. This is in line with our earlier published observation[14] that a smaller proportion of offspring in the fish oil group had been given a hospital asthma diagnosis. The main finding of the present study is that fish oil supplementation not only seems to have prophylactic potential on severe asthma, but also on mild to moderate asthma, which is a finding of high public health relevance.

There are three possible explanations for our findings: 1) fish oil reduced the risk of asthma, 2) olive oil increased the risk of asthma), or 3) a combination of those two effects. We believe that the first explanation is most likely. The biological pathways underlying the positive impact of fish oil could be explained by a reduced T_H2 immune response in the offspring exposed to n-3 polyunsaturated fatty acids in fetal life. Fatty acids cross the placenta[18] and EPA and DHA may directly affect the development of the fetal immune and respiratory systems by influencing cell signalling and gene expression; competing with arachidonic acid to produce less potent eicosanoids; and via the production of anti-inflammatory resolvins.[19-21] Alternatively, fatty acids may act indirectly by skewing the maternal immune system towards a T_H2 response, resulting in an increased production of inflammatory immune cells that cross the placenta and influence the fetal immune system development.[22] Although olive oil provides linoleic acid, which through elongation and desaturation processes can form arachidonic acid, which in turn is a precursor for several inflammatory mediators, we do not believe that the given dosage of 4 g daily could result in any notable effects. In fact, the amount of olive oil in the placebo group provided <3% of average daily intake of linoleic acid at that time in Denmark.[10] In contrast, the dosage of EPA and DHA of 2.7 g daily resulted in a ~10 fold increase in that nutrient.

As shown in the supplementary material, offspring of mothers in the no oil group were remarkably similar to offspring in the fish oil group in terms of the occurrences of asthma and allergic disease. We believe this intriguing finding may be the result of contamination bias. All participants were informed about the study hypothesis relating 2.7 g n-3 PUFA supplementation in pregnancy with a reduced risk of preterm birth, and it is possible that those who subsequently did not receive any capsules as part of the trial have self-supplemented or increased their intake of fish during the study

period. Data collected post-partum asking about changes in fish intake and self-supplementation revealed such increases were indeed more frequent in the no oil group compared to the olive oil group. It can be argued that a passive intervention informing women about the potential beneficial effects of fish oil may be as effective as actual fish oil supplementation, at least in a group of motivated pregnant women.

A major asset of the national prescription and patient registers is that they cover all citizens in Denmark. This becomes particularly potent in a randomised setting like ours. Since all individuals are followed, analyses could be performed as intention-to-treat and the full scientific advantage of the randomised design for making causal inferences retained, even in a long-term perspective. As a consequence of this, the observed differences in asthma occurrences across trial groups can with great certainty be ascribed to a causal effect of the fish oil compared to olive oil regimens.

The two registers have their own characteristic patterns of misclassification. Milder asthma cases solely treated with asthma medicine prescribed by general practitioners or paediatricians but never admitted to hospital confers a higher sensitivity to the prescription register. This may however be at the cost of specificity, since asthma medication may be prescribed in order to clarify a suspected asthma diagnosis. We tried to avoid the latter problem by requiring at least two prescriptions for beta-2-agonists and steroids to be counted as a case based on medications. The fact that analyses based on the two different registers agreed in their conclusion, despite different underlying error sources, adds further credibility to the belief that the intervention group and placebo group truly did differ in asthma occurrences. Any misclassification with respect to identifying children who truly had or did not have asthma is likely to have occurred to a similar extent across the trial groups, and will have tended to attenuate our measures of association rather than create spurious ones. Additional support comes from the self-reported outcomes at 18-19 years of age. According to these, the odds of asthma tended to be lower in the fish oil compared the olive oil group, although the differences were significant only for current use of asthma medication.

In contrast, no impact of fish oil was observed on the lung function measures. The clinical follow-up of offspring required active participation, which implied a certain degree of differential attrition.

This could potentially have introduced biased estimates if non-participants had a different disease risk than participants, but there were no differences in disease occurrences according to register outcomes that were available for everyone in the cohort (Table II), making selection bias less likely to explain the results. The null results for lung function may rather be due to an influence of current asthma or hayfever medication use when assessing asthma at the clinical examination at age 18-19 years. Although we asked offspring not to use any medication on the day of the examination, the use of long-acting medications especially inhaled corticosteroid in well-treated offspring may have prevented us from detecting any associations with the lung function outcomes. Furthermore, we observed no significant impact on parameters reflection sensitization such as total and specific IgE. Remarkably, the probability of being prescribed allergic rhinitis medication tended to be lower in the fish oil group compared to the olive oil group, and the effect of fish oil seemed to be strongest on the allergic asthma phenotype, which may still leave uncertainty about a role of allergic sensitization in mediating a possible protective effect of fish oil on asthma.

Medication data regarding the first 5 years of follow-up in the prescription register were lacking, obstructing us from investigating common phenotypes of allergic disease in early childhood. In consequence, we are not able to compare our results to other RCTs in the field that have assessed allergic outcomes up to 3 years of age. These RCTs have, for the most part, shown favourable effects on immunological responses at birth[3-5] and sensitization[23;24] during the first 12 and 24 months, respectively, but no effects on clinical outcomes such as eczema, food reactions, or wheezing and asthma. Perhaps this early effect on the immune response and sensitization may later establish itself clinically, as suggested by our results. Long-term follow-up of these RCTs will therefore be important.

In conclusion, results from this randomised controlled trial showed that children whose mothers had taken fish oil in third trimester of pregnancy had a reduced probability of having had asthma medication prescribed and of receiving an asthma diagnosis during their childhood and early adulthood compared with children whose mothers had received olive oil. These results are compatible with the hypothesis that supplementation with n-3 PUFA late in pregnancy has prophylactic potential in preventing asthma in the offspring. More generally, our results support the

hypothesis that the intrauterine environment is critical for the development of asthma, not only in childhood, but also in a long-term perspective.

Reference List

(1) Duijts L. Fetal and infant origins of asthma. *Eur J Epidemiol* 2012; 27(1):5-14.

(2) Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta* 2015; 1851(4):469-484.

(3) Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG et al. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003; 112(6):1178-1184.

(4) Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG et al. Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. *Clin Exp Allergy* 2003; 33(4):442-448.

(5) Krauss-Etschmann S, Hartl D, Rzehak P, Heinrich J, Shadid R, Del CR-T et al. Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women. *J Allergy Clin Immunol* 2008; 121(2):464-470.

(6) Furuhjelm C, Warstedt K, Larsson J, Fredriksson M, Bottcher MF, Falth-Magnusson K et al. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. *Acta Paediatr* 2009; 98(9):1461-1467.

(7) Olsen SF, Osterdal ML, Salvig JD, Mortensen LM, Rytter D, Secher NJ et al. Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am J Clin Nutr* 2008; 88(1):167-175.

(8) Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. *Scand J Public Health* 2011; 39(7 Suppl):30-33.

(9) Hansen S, Strom M, Maslova E, Mortensen EL, Granstrom C, Olsen SF. A comparison of three methods to measure asthma in epidemiologic studies: results from the danish national birth cohort. *PLoS One* 2012; 7(5):e36328.

(10) Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS et al. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet* 1992; 339(8800):1003-1007.

(11) Sorensen JD, Olsen SF, Pedersen AK, Boris J, Secher NJ, FitzGerald GA. Effects of fish oil supplementation in the third trimester of pregnancy on prostacyclin and thromboxane production. *Am J Obstet Gynecol* 1993; 168(3 Pt 1):915-922.

(12) van Houwelingen AC, Sorensen JD, Hornstra G, Simonis MM, Boris J, Olsen SF et al. Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy. *Br J Nutr* 1995; 74(5):723-731.

(13) Boris J, Jensen B, Salvig JD, Secher NJ, Olsen SF. A randomized controlled trial of the effect of fish oil supplementation in late pregnancy and early lactation on the n-3 fatty acid content in human breast milk. *Lipids* 2004; 39(12):1191-1196.

(14) Kildemoes HW, Sorensen HT, Hallas J. The Danish National Prescription Registry. *Scand J Public Health* 2011; 39(7 Suppl):38-41.

(15) Moth G, Vedsted P, Schiotz P. Identification of asthmatic children using prescription data and diagnosis. *Eur J Clin Pharmacol* 2007; 63(6):605-611.

(16) Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; 16:5-40.:5-40.

(17) Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8(3):483-491.

(18) Haggarty P. Placental regulation of fatty acid delivery and its effect on fetal growth--a review. *Placenta* 2002; 23 Suppl A:S28-S38.

(19) Calder PC, Kremmyda LS, Vlachava M, Noakes PS, Miles EA. Is there a role for fatty acids in early life programming of the immune system? *Proc Nutr Soc* 2010; 69(3):373-380.

(20) Prescott SL, Dunstan JA. Prenatal fatty acid status and immune development: the pathways and the evidence. *Lipids* 2007; 42(9):801-810.

(21) Serhan CN, Arita M, Hong S, Gotlinger K. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* 2004; 39(11):1125-1132.

(22) Warner JO. The early life origins of asthma and related allergic disorders. *Arch Dis Child* 2004; 89(2):97-102.

(23) Furuhjelm C, Warstedt K, Fageras M, Falth-Magnusson K, Larsson J, Fredriksson M et al. Allergic disease in infants up to 2 years of age in relation to plasma omega-3 fatty acids and maternal fish oil supplementation in pregnancy and lactation. *Pediatr Allergy Immunol* 2011; 22(5):505-514.

(24) Palmer DJ, Sullivan T, Gold MS, Prescott SL, Heddle R, Gibson RA et al. Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. *BMJ* 2012; 344:e184.

Competing interests

All authors declare no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work.

Author contributions

The authors' responsibilities were as follows – SFO was responsible for the original pregnancy trial and initiated the follow-up of the offspring. SH and SFO: study concept and design; CG: data preparation and management; DR, BHB, SFO, TBH, RD, HJH designed the follow-up study, SH: conducted the statistical analyses and drafted the manuscript; SH, MS, EM, RD, HJH, DR, BHB, TBH, CG, TIH, JEC, AL, SFO: contributed critical advice and revisions of the manuscript; SFO: funding; SH and SFO: acquisition of data and responsibility for the entire contents of the manuscript. All authors had full access to study data. None of the authors had a conflict of interest.

Figure legends

Figure 1. Participation flow in the Aarhus Trial

Figure 2. 24-year follow-up of offspring from a randomized controlled trial (n=396) with fish oil supplementation in pregnancy. Kaplan-Meier survival curves show occurrences of asthma discharge diagnoses in the national patient register (2a), and asthma (2b) and allergic rhinitis medication use (2c), respectively, in the national prescription registry, stratified by randomization group.

Figure 3. Odds ratios (ORs), ratios between geometric means, and mean differences with 95% confidence intervals of self-reported (3a) and clinical outcomes (3b and 3c) comparing offspring in the fish oil group to the olive oil group

Supplementary Table I. Hazard ratios (HRs) and 95% CIs of register outcomes of asthma and allergic rhinitis in the fish oil group and no oil group relative to the olive oil group.

Registry-based outcomes (n=522)	**HR (95% CI)**
Asthma discharge diagnosis	
Fish oil	0.31 (0.13-0.75)
No oil	0.24 (0.07-0.86)
Olive oil	1.00
Asthma medication	
Fish oil	0.54 (0.32-0.90)
No oil	0.43 (0.22-0.85)
Olive oil	1.00
Allergic rhinitis medication	
Fish oil	0.70 (0.47-1.05)
No oil	0.66 (0.40-1.08)
Olive oil	1.00

Hazard ratios calculated using Cox regression analysis.

Supplementary Table II. Odds ratios (ORs) and 95% CIs of self-reported outcomes of asthma and hayfever in the fish oil group and no oil group relative to the olive oil group.

Self-reported outcomes (n=382)	**OR (95% CI)**	
	Crude	**Adjusted**
Lifetime doctor diagnosis of asthma		
Fish oil	0.52 (0.25-1.06)	0.50 (0.24-1.04)
No oil	0.44 (0.17-1.12)	0.41 (0.16-1.05)
Olive oil	1.00	1.00
Current asthma medication use		
Fish oil	0.35 (0.12-1.02)	0.34 (0.12-0.99)
No oil	0.37 (0.10-1.42)	0.34 (0.09-1.30)
Olive oil	1.00	1.00
Lifetime doctor diagnosis of hayfever		
Fish oil	0.64 (0.32-1.28)	0.64 (0.32-1.29)
No oil	1.00 (0.47-2.13)	1.00 (0.47-2.15)
Olive oil	1.00	1.00
Current hayfever symptoms		
Fish oil	0.72 (0.43-1.21)	0.70 (0.42-1.19)
No oil	0.96 (0.52-1.75)	0.92 (0.50-1.69)
Olive oil	1.00	1.00

Odds ratios calculated using logistic regression. Adjusted for offspring sex and maternal smoking.

Supplementary Table III. Odds ratios (ORs), mean differences (β), or ratios between geometric means in clinical outcomes of allergic sensitization, lung function, and ECP in the fish oil group and no oil group relative to the olive oil group.

Outcomes from clinical examination (n=243)	**OR, β estimates*, or ratios between geometric means** (95% CI)**	
	Crude	**Adjusted**
Specific IgE > 0.35 kU_A/L		
Fish oil	0.67 (0.36-1.25)	0.69 (0.37-1.28)
No oil	0.70 (0.35-1.41)	0.74 (0.36-1.50)
Olive oil	1.00	1.00
FEV_1/FVC (%)*		
Fish oil	0.14 (-2.01, 2.28)	-0.14 (-2.21, 1.92)
No oil	0.40 (-2.04, 2.83)	0.19 (-2.16, 2.54)
Olive oil	0.00	0.00
FEV_1% predicted (%)*		
Fish oil	-1.40 (-4.57, 1.77)	-1.33 (-4.51, 1.87)
No oil	-2.06 (-5.66, 1.53)	-1.90 (-5.54-1.74)
Olive oil	0.00	0.00
Total IgE (kU_A/L)**		
Fish oil	1.13 (0.71, 1.80)	1.16 (0.73-1.86)
No oil	0.75 (0.45, 1.29)	0.80 (0.47-1.36)
Olive oil	1.00	1.00
ECP (µg/L)**		
Fish oil	1.04 (0.86, 1.27)	1.03 (0.85-1.26)
No oil	1.04 (0.85, 1.28)	1.00 (0.80-1.26)
Olive oil	1.00	1.00

Odds ratios calculated using logistic regression; β estimates and ratios between geometric means calculated using linear regression. Adjusted for offspring sex and maternal smoking.

Supplementary Table IV. Odds ratios (ORs), mean differences (β), or ratios between geometric means of self-reported and clinical outcomes of asthma, hay fever, allergic sensitization, lung function, total IgE, and ECP in the fish oil group relative to the olive oil group.

Outcomes from clinical examination (n=180)	**OR, β estimates*, or ratios between geometric means** (95% CI)**	
	Crude	**Adjusted**
Lifetime doctor diagnosis of asthma		
Fish oil	0.52 (0.25-1.06)	0.50 (0.24-1.04)
Olive oil	1.00	1.00
Current asthma medication use		
Fish oil	0.35 (0.12-1.02)	0.34 (0.12-0.99)
Olive oil	1.00	1.00
Lifetime doctor diagnosis of hayfever		
Fish oil	0.64 (0.32-1.28)	0.64 (0.32-1.29)
Olive oil	1.00	1.00
Current hayfever symptoms		
Fish oil	0.72 (0.43-1.21)	0.70 (0.42-1.19)
Olive oil	1.00	1.00
Total IgE (kU_A/L)**		
Fish oil	1.13 (0.72, 1.77)	1.16 (0.75-1.83)
Olive oil	1.00	1.00
ECP (µg/L)**		
Fish oil	1.04 (0.86, 1.27)	1.03 (0.85-1.26)
Olive oil	1.00	1.00
Specific IgE > 0.35 kU_A/L		
Fish oil	0.67 (0.36-1.25)	0.69 (0.37-1.28)
Olive oil	1.00	1.00
FEV_1/FVC (%)*		
Fish oil	0.14 (-1.82, 2.10)	-0.04 (-1.96, 1.89)
Olive oil	0.00	0.00
FEV_1% predicted (%)*		
Fish oil	-1.40 (-4.60, 1.81)	-1.21 (-4.43, 2.01)
Olive oil	0.00	0.00

Odds ratios calculated using logistic regression; β estimates and ratios between geometric means calculated using linear regression. Adjusted for offspring sex and maternal smoking.

Supplementary Table V. Hazard ratios (HRs) and 95% CIs of register outcomes of asthma and allergic rhinitis in the fish oil group relative to the olive oil group adjusted for birth weight and gestational age.

	Crude	Adjustment for birth weight	Adjustment for gestational age
Registry-based outcomes (n=394)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Asthma discharge diagnosis			
Fish oil	0.31 (0.13-0.75)	0.33 (0.14-0.81)	0.31 (0.13-0.76)
Olive oil	1.00	1.00	1.00
Asthma medication			
Fish oil	0.54 (0.32-0.90)	0.56 (0.33-0.94)	0.55 (0.33-0.93)
Olive oil	1.00	1.00	1.00
Allergic rhinitis medication			
Fish oil	0.70 (0.47-1.05)	0.69 (0.46-1.04)	0.70 (0.47-1.06)
Olive oil	1.00	1.00	1.00

Hazard ratios calculated using Cox regression.